

THE JUVENILE HORMONE. I. ENDOCRINE ACTIVITY OF THE CORPORA ALLATA OF THE ADULT CECROPIA SILKWORM

CARROLL M. WILLIAMS¹

The Biological Laboratories, Harvard University, Cambridge 38, Massachusetts

The endocrine role of the corpora allata of insects was discovered by V. B. Wigglesworth (1934, 1936) over twenty years ago. In a series of simple and decisive experiments on *Rhodnius* he showed that the corpora allata secrete a "juvenile hormone" which opposes metamorphosis. In these early studies Wigglesworth also recognized that the corpora allata undergo pronounced changes in endocrine activity during the course of metamorphosis; namely, that they are active in the immature nymph, inactive in the mature nymph just prior to metamorphosis, and active again in the adult insect after metamorphosis. Subsequently, the general validity of these conclusions has been confirmed repeatedly and found to apply to both hemi- and holometabolous insects (for review, see Wigglesworth, 1954, pages 56-64).

During the past twelve years, in the course of studies of the metamorphosis of the Cecropia silkworm, the juvenile hormone has necessarily been an object of detailed attention. While confirming the essential elements in Wigglesworth's theory, the study has helped to resolve certain persistent mysteries and, more recently, has pointed the way to the successful extraction and purification of the hormone itself. This first of a series of communications is concerned with the endocrine activity of the corpora allata of the adult moth.

MATERIALS AND METHODS

1. *Experimental animals*

The experiments were performed on Cecropia, Cynthia, and Polyphemus silkworms. Taxonomists continue to amuse themselves by changing the generic and specific names of these Saturniids. What began as *Phalaena cecropia* became *Samia cecropia*, then *Platysamia cecropia*, and now *Hyalophora cecropia* (Michener, 1952). The Cynthia silkworm, known throughout the world as *Philosamia cynthia*, was changed to *Samia walkeri*, and then back to *Samia cynthia*. *Telea polyphemus* is now *Antheraea polyphemus*. As in the analogous cases discussed by Wald (1952, page 339), the "common names" have escaped the attention of taxonomists and have remained firm and unchanging. Therefore, the common names will be used routinely in the present reports.

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Cecropia silkworms were reared under nylon nets on wild-cherry trees. Polyphemus were reared on oak or maple; Cynthia, on cherry or ailanthus or purchased from dealers. The cocoons were harvested and stored as previously described (Williams, 1946a; Shappirio and Williams, 1957).

2. *Surgical procedures*

Experimental animals must be deeply anesthetized during surgical procedures. We use carbon dioxide for this purpose and with mixtures of air and carbon dioxide have maintained pupae anesthetized for as long as one month without injury. Groups of animals are placed in a capped, flat-bottom Büchner funnel and exposed for about twenty minutes to a slow stream of carbon dioxide from a compressed cylinder. The gas is bubbled through water *en route* to the funnel. The animals are flaccid when fully anesthetized, and one can no longer elicit any movements of the abdominal segments.

Surgical procedures are performed in a second Büchner funnel (diameter 11 cm., height 3 cm.) which is mounted flush on the top of the operating bench. A slow stream of carbon dioxide is bubbled through water and passed through the bottom of the uncovered funnel. Carbon dioxide, being heavier than air, fills the cavity of the funnel and maintains a continuous anesthesia during the surgical procedure (Williams, 1946b).

Operations are carried out under the low magnification of the dissecting microscope, making use of $9\times$ oculars and 0.7, 1, or $2\times$ objectives. The foot of the microscope is removed and the vertical pillar permanently attached to the operating bench on the distal side of the funnel. A hinged-arm permits the microscope to scan the entire diameter of the funnel. In order to leave both hands free, the microscope is equipped with a foot-focusing device (designed and built by Mr. Robert Chapman of the Harvard Biological Laboratories). Illumination is provided by a 6-volt microscope lamp (Zeiss "Osram") attached to and moving with the microscope. The lamp is equipped with an infra-red filter.

Anesthetized animals are transferred to the carbon dioxide-filled funnel for the surgical procedure. They are then returned to air, placed in individual numbered glass containers ("creamers"), and stored in a room having a controlled humidity of sixty per cent and a temperature of 25° C.

Dissecting instruments consist of the following: watchmaker's forceps (Dumont "rustless"; two of No. 3 and two of No. 5); a scalpel (Bard-Parker No. 3 handle with a No. 11 detachable blade); stainless iris scissors curved on the flat and closing to the tip; several forms of stainless steel iridectomy and micro-scissors; a stainless steel dental probe; a 5-ml. hypodermic syringe filled with insect Ringer and capped with a 25-gauge needle.

Prior to each group of operations the instruments are briefly rinsed in seventy per cent ethanol and wiped dry. Rigorous asepsis is unnecessary because the blood of the silkworms apparently contains an anti-bacterial substance that protects it from the ordinary contaminants. However, it fails to protect from insect pathogens and no diseased insect should be operated upon with the same instruments or even in the same room.

Healthy pupae can withstand almost any degree of surgery provided that a few crystals of the potent anti-tyrosinase, phenylthiourea, are placed in the operat-

ing field. We routinely use an equal part mixture of phenylthiourea (twice recrystallized from hot 95 per cent ethanol) and streptomycin sulphate, the two having been ground together in a mortar and stored in a capped vial in the refrigerator. Small amounts of the powder are removed and discarded within two days after being placed at room temperature.

Ephrussi-Beadle Ringer's solution is utilized containing 7.5 gm. NaCl, 0.35 gm. KCl, and 0.21 gm. CaCl₂, per liter of distilled water. The stock solution is brought to a boil, capped, and stored in the dark under refrigeration. Fungal contamination of physiological solutions, especially those containing bicarbonate, is a common source of difficulty when solutions are stored at room temperature.

Excised tissues and organs are transferred to small depression dishes made of black glass and filled with Ringer. Black plastic bottle-caps are also satisfactory for this purpose. Dissections of sacrificed animals are performed in a glass Petri dish which fits snugly into the cavity of the Büchner funnel. Plasticine is pressed into the bottom of the dish to receive short stainless steel pins. The dish is filled with Ringer and the dissection performed with the animal spread and pinned under the solution.

After surgical procedures on surviving pupae, Ringer's solution is added from a hypodermic syringe so that the blood is flush with the surface of the cuticle. The area of excised cuticle is then capped by a plastic window of appropriate size. The latter is punched or cut with scissors from cellulose acetate cover slips ("Turtox," thickness 1 or 2). The window is sealed in place with paraffin wax which is melted in an alcohol lamp and transferred with a curved needle or drawing pen. The melted wax adheres to the cuticle and the underside of the rim of the plastic slip provided that both are dry. The operating field is thereby equipped with a transparent window which permits one to look inside the living animal.

3. *Excision of pupal corpora allata and corpora cardiaca*

An anesthetized pupa is placed in a plasticine cradle in the bottom of the carbon dioxide-filled funnel. The cuticle of the facial region is first removed. For this purpose a scalpel incision is made through the integument on each side of the face. The two cuts are joined by a transverse cut and the rectangle of cuticle is grasped with forceps and pulled free from its attachment at the base of the legs. The insect's abdomen is then pressed forward with plasticine and held in this position so that the blood fills, but does not overflow, the operating field. The naked epidermis is grasped with forceps, split down the middle, and trimmed free with scissors. The brain is thereby exposed. This is pressed down in the field to reveal the tiny corpus allatum-corpora cardiaca complex on each side. The complexes are dorso-lateral to the brain and attached on each side to a large tracheal trunk at this position (see Figure 1). A pair of tiny nerves emerges from the posterior face of each brain hemisphere and passes to the corpora cardiaca on that side. These nerves are very delicate and difficult to see in a dissection of this type.

By means of forceps the connections between glandular complex and the adjacent trachea are broken, and the complex transferred to Ringer's solution in a black dish. Alternatively, the tracheal segment can be excised with iridectomy scissors and removed along with the glandular complex.



FIGURE 1.

4. *Excision of adult corpora allata*

The moth is anesthetized and its head dipped momentarily into seventy per cent ethanol to wet the scales and hairs. The head is then cut off with scissors and placed in Ringer's solution. (The headless moth will continue to live for approximately the normal life-span of 7 to 10 days at 25° C.)

The antennae are excised at their bases. Then with fine scissors the head is cut along the dorsal midline from its posterior margin to the mouth parts. The head is then spread apart with forceps and pinned under Ringer. The pair of corpora allata-corpora cardiaca complexes is attached to the aorta just behind the brain. The brain is split in the midline to expose the aorta. The glandular complexes can now be broken free from the rear of the brain and transferred to a black dish by grasping the aorta with forceps.

Under the favorable conditions of illumination in the black dish, one can recognize the corpus cardiacum; it is attached by short nerves to the much larger corpus allatum. The latter is ordinarily flattened or wedge-shaped and subdivided into a number of lobes and lobules. If necessary, the glandular complex may now be subdivided into its constituent parts by breaking the nerves between corpus cardiacum and corpus allatum.

5. *Isolation of pupal abdomens*

This procedure has already been described for the *Cecropia* silkworm (Williams, 1947). The principal difficulty is to isolate the terminal abdominal segments without puncturing the fluid-filled midgut. This difficulty is circumvented by the use of the *Cynthia* silkworm. In this species the midgut contains only a solid, rod-like mass. Therefore the perforation of the midgut is inconsequential. The pupa is transected just behind the metathorax with a single transverse cut of a sharp razor blade. The abdomen is then supported with the cut surface facing upward. Crystals of the phenylthiourea-streptomycin mixture are spread in the wound, and Ringer's solution is added to fill the cavity of the abdomen. The wound is then capped with a plastic slip in which a central hole has been punched. The plastic is sealed in place with melted wax. Ringer is finally added *via* the central hole to replace all air, and the hole itself sealed with wax.

RESULTS

1. *Role of the corpora allata in adult development and sexual maturation*

The pair of corpora allata-corpora cardiaca complexes was removed from each of a series of twenty chilled male or female *Cecropia* pupae *via* the facial approach. The integumentary defect was capped and sealed with a plastic window, and the animals placed at 25° C.

Adult development was initiated after about two weeks and proceeded in synchrony with the time-table for the normal development of *Cecropia* at 25° C. (Schneiderman and Williams, 1954). The moths, emerging after three weeks

FIGURE 1. Brain and corpora allata of the *Cecropia* silkworm are shown in cutaway views of the head of larva (*top*), pupa (*middle*), and adult (*bottom*). The corpora allata are the two small bodies attached by tiny nerves to the back of the brain. (This figure is used with the permission of *Scientific American*.)

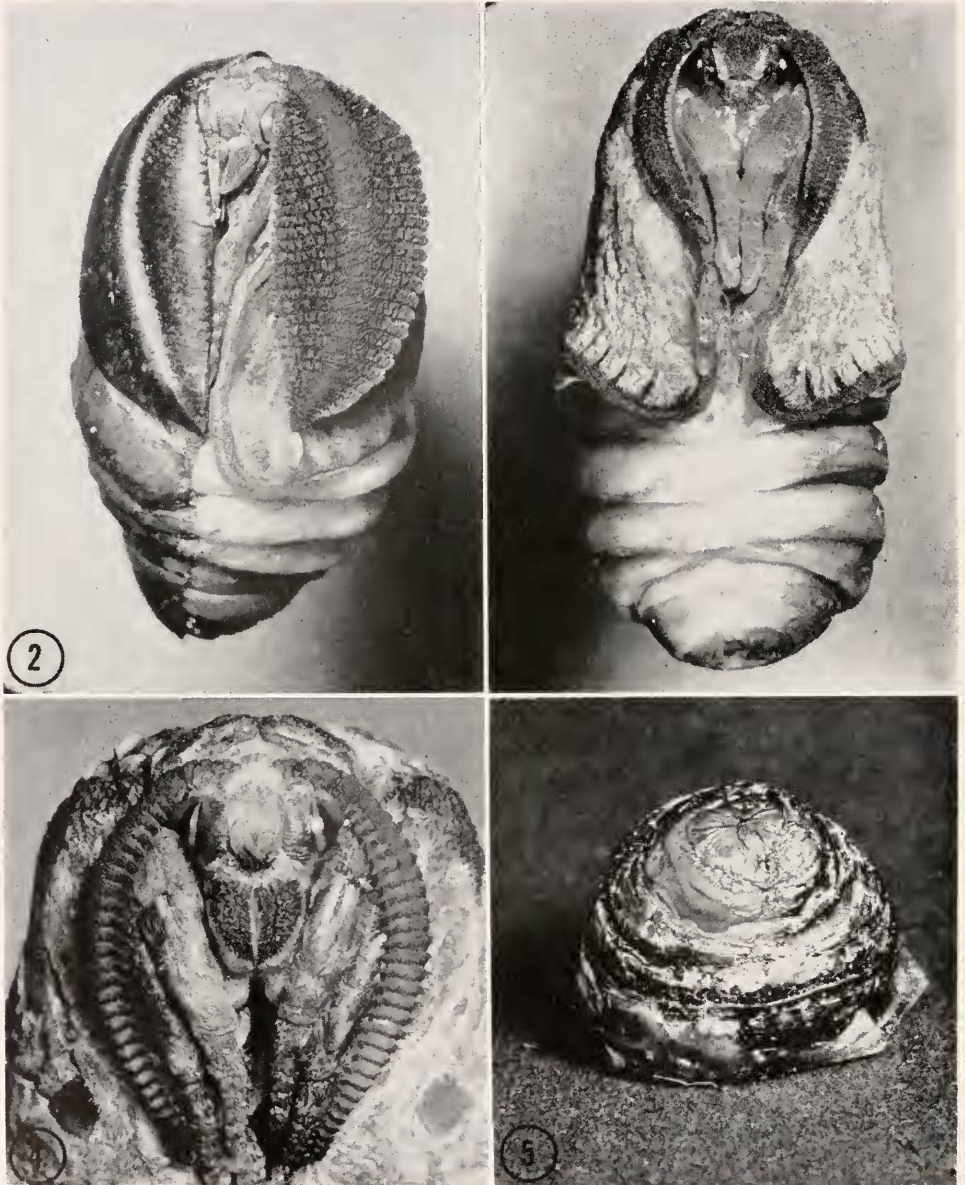


FIGURE 2. After receiving implants of three pairs of corpora allata of adult *Cecropia*, the *Polyphemus* pupa, here illustrated, has transformed into a second pupal stage. (See right side of preparation where the old pupal cuticle has been trimmed away.)

FIGURE 3. This *Cecropia* pupa received implants of two pairs of adult *Cecropia* corpora allata. Development has given rise to a mixture of pupa and adult. (The old pupal cuticle has been completely removed.)

of adult development, could not be distinguished from un-operated individuals. The females deposited a normal complement of eggs and both sexes survived for the customary period of 7 to 10 days at 25° C.

The absence of corpora allata was confirmed in dissections of many of these moths. All the internal organs, including the gonads, showed full and complete development. The abdomens of females were packed with ripe eggs, and the males showed normal spermatogenesis.

The experiment was repeated on a series of six male and six female pupae to produce moths lacking corpora allata. The two sexes were cross-mated and each of the six females was allowed to oviposit in a paper bag. A normal number (150-225) of eggs was collected from each female. These were placed under large nylon nets and the larvae reared to maturity on wild-cherry leaves. No deviation from normal development could be detected.

These experiments show that the corpora allata play no evident role in the transformation of the pupa into an adult *Cecropia* or in the gonadal function of the adult itself.

2. *Endocrine activity of adult corpora allata*

In the absence of any obvious function of the corpora allata of adult *Cecropia*, it is paradoxical to find that the glands, when excised and tested for endocrine activity, are more active in the moth than at any other stage in the life history (Williams, unpublished data). This fact was discovered eleven years ago in the course of an experiment performed for other purposes. It happened by chance that a pair of adult corpora allata was implanted into a brainless diapausing *Cecropia* pupa. Ten days later, the host showed the termination of diapause and the initiation of development. This result would have been puzzling in a normal diapausing pupa; in a brainless diapausing pupa it was incomprehensible.

Even more puzzling was the character of the development which then took place. Within two weeks the brainless pupa transformed, not into a moth, but into a bizarre creature in which large areas of pupal cuticle had been freshly formed (see Figs. 3 and 4). The animal, in short, was a mosaic of pupal and adult characteristics (Williams, 1952b).

During the past eleven years this result has been duplicated on numerous occasions. The experimental series includes fifty-one brainless *Cecropia* pupae which received one to three pairs of corpora allata-corpora cardiaca complexes derived from male or female *Cecropia* moths. As shown in Table I a total of twelve individuals (23 per cent) showed the result just described. The residual 77 per cent showed no effect of the implantation and continued to diapause. But the twelve positive experiments were of sufficient interest in themselves. Not only

FIGURE 4. Pupal-adult monstrosity after implantation of adult corpora allata. Note the pupal cuticle on head, palps, and antennae. However, the wings show scale-covered adult cuticle and the eyes show considerable adult development.

FIGURE 5. This isolated pupal abdomen received implants of adult corpora allata, plus an injection of ecdysone. The tip of the old pupal cuticle has been torn away to reveal a second pupal abdomen that has formed.

TABLE I

Tests of adult corpora allata-corpora cardiaca complexes in brainless diapausing pupae*

Adult donors	Brainless hosts	Normal development	Mixed development	No development
Cecropia	Cecropia	0	12	39
Cecropia	Cynthia	0	2	2
Cecropia	Polyphemus	0	2	1
Cynthia	Cynthia	0	0	5
Cynthia	Cecropia	0	0	20
Polyphemus	Polyphemus	0	2	0
Polyphemus	Cecropia	0	4	10
Totals		0	22	77

* One to three pairs of complexes from male or female moths were implanted into each brainless pupa.

had the implants caused the formation of mixtures of pupa and adult; seemingly, they also had substituted for the brain and provoked the termination of diapause.

As shown in Table I, this result was duplicated when corpora allata of adult male or female *Cecropia* were implanted into brainless diapausing pupae of *Cynthia* or *Polyphemus*. Here again, a certain percentage of animals terminated diapause and developed into pupal-adult mixtures.

The corpora allata-corpora cardiaca complexes of male and female *Cynthia* and *Polyphemus* moths were also tested. The three species seem to differ among themselves in the endocrine activity of the adult corpora allata. For example, the corpora allata of adult *Cynthia* gave negative tests in all twenty-five preparations. By contrast, the glands of adult *Polyphemus* gave positive tests in six of sixteen preparations. Moreover, when used as recipients of implants, brainless *Polyphemus* pupae seemed to have a lower developmental threshold than the other two species, for four of five individuals gave a positive reaction to the implantation of adult corpora allata. In retrospect, *Polyphemus* appears to be the animal of choice for experiments of this type.

In the far more numerous tests of *Cecropia* corpora allata, the conditions of the experiment were subjected to minor variations in the hope of recruiting a positive response in a larger proportion of individuals. By increasing the number of implanted glands from one to two or three pairs, little additional effect was realized. However, the developmental response was markedly enhanced when the host animals were placed at 15 or 20° C. rather than at 25° C. after the implantation of corpora allata. It was also observed that the experimental animals which developed at the lower temperature retained a far larger proportion of pupal characters than in similar animals developing at 25° C.

3. *Inactivity of corpora cardiaca*

In the experiments just considered, the adult corpora allata were implanted together with the attached corpora cardiaca. However, in thirty-five additional preparations, the corpora allata were carefully dissected from the attached corpora cardiaca and then implanted into brainless diapausing pupae.

TABLE II

Tests of adult corpora allata (minus corpora cardiaca) in brainless diapausing pupae*

Adult donors	Brainless hosts	Normal development	Mixed development	No development
Cecropia	Cecropia	0	4	23
Cynthia	Cynthia	0	0	2
Cynthia	Cecropia	0	1	3
Polyphemus	Polyphemus	0	1	0
Polyphemus	Cecropia	0	0	1
Totals		0	6	29

* One to three pairs of corpora allata from male or female moths were implanted into each brainless pupa.

The results, recorded in Table II, were substantially the same as those observed in the previous experiments. Once again, a certain low percentage of brainless animals terminated diapause and transformed into pupal-adult monstrosities.

The inactivity of implanted corpora cardiaca was further confirmed in fourteen experiments in which adult corpora cardiaca were freed from corpora allata and tested, as such, in brainless diapausing pupae. No developmental response was obtained even when as many as ten pairs of adult corpora cardiaca were implanted. Indeed, in the course of twelve years of experimentation, we have never detected any trace of developmental response after the implantation of corpora cardiaca of larvae, pupae, or adults.

For present purposes it is necessary to conclude that the developmental reactions under consideration are attributable to the adult corpora allata *per se*. This implies that in a certain proportion of individuals the adult corpora allata have two effects: they first promote the initiation of adult development; they then prevent the transformation of the pupa into a normal adult moth.

4. *Effects of brain implantation*

As noted in Tables I and II, the vast majority of brainless *Cecropia* pupae continued to diapause when implanted with adult corpora allata. In all of these preparations the implants gave the impression of being inert. The true state of affairs is suggested by the following experiment:

Two pairs of adult corpora allata were implanted into each of five brainless *Cecropia* pupae. Six weeks later the pupae showed no change from their condition at the outset. Two brains of previously chilled *Cecropia* pupae were implanted at this time to cause the initiation of development. The latter gave rise to creatures showing large areas of pupal cuticle. In effect, the initiation of development unmasked the endocrine activity of the previously implanted corpora allata. Further information was provided by the following experiment:

Two pairs of adult *Cecropia* corpora allata were implanted under a facial window in each of two brainless diapausing *Cecropia* pupae. One month later the implants were removed and the pupae caused to develop by the injection of 125 μ g. of a

purified extract of prothoracic gland hormone (ecdysone).² Both individuals transformed into moths which retained large areas of pupal cuticle.

This experiment shows that the presence of the brain is not necessary for the secretion of juvenile hormone by adult corpora allata. In the absence of the initiation of adult development, the implants had built up a substantial titer of juvenile hormone. But the host could not signal this fact until its development was brought about by ecdysone.

5. Experiments on isolated pupal abdomens

Eight abdomens were isolated from diapausing *Cecropia* pupae. Preparations of this type remain in permanent diapause unless provided with ecdysone by injection (Williams, 1954), or by the implantation of active prothoracic glands, or by the implantation of inactive prothoracic glands plus active brains (Williams, 1952a). In the present experiment efforts were made to evoke a developmental response of isolated abdomens by the implantation of adult corpora allata—either alone, or in conjunction with brains, prothoracic glands, or injections of ecdysone.

TABLE III
Effects of implantations into isolated abdomens of diapausing cecropia

Abdomen no.	Implant	Result
1415	1 pr. adult C.C. + C.A.	No development
1447	1 pr. adult C.C. + C.A.	No development
2123	3 pr. adult C.C. + C.A.	No development
2090	5 pr. adult C.C. + C.A.	No development
2212	2½ pr. adult C.C. + C.A. plus 2 chilled pupal brains	No development
1515	1 pr. adult C.C. + C.A. plus 2 pr. prothoracic glands of diapausing pupae	No development
2109	3½ pr. adult C.C. + C.A. plus 4 pr. prothoracic glands of diapausing pupae	Molted to form second pupal abdomen
9320	2 pr. adult C.A. (-C.C.) plus 25 µg. of crystalline ecdysone	Molted to form second pupal abdomen

Table III summarizes the several types of preparations. It is of particular interest and importance to note that no development took place when the abdomens received only adult corpora allata. We have checked this finding in twelve additional experiments performed on isolated *Cynthia* abdomens; in this case the pupal abdomens were distributed at 15, 20, and 25° C. after the implantation of two to five pairs of corpora allata derived from adult *Cecropia* or *Polyphemus*. In short, no trace of development was ever observed in response to the implantation of adult corpora allata *per se*. The same negative result was also recorded in an experiment where adult corpora allata were implanted along with active brains.

The preparation numbered 9320 in Table III is of particular interest. Here, two pairs of adult corpora allata were implanted into an isolated abdomen. A

² I am indebted to Dr. Peter Karlson for supplying highly purified preparations of ecdysone.

month later 25 μ g. of crystalline ecdysone were injected. Development began within two days. Within the following ten days the pupal abdomen transformed and molted into a second pupal abdomen (see Figure 5). This result was duplicated in two additional experiments utilizing *Cynthia* abdomens. It is clear that ecdysone is the prime-mover in the developmental response and that the juvenile hormone is inactive in the absence of ecdysone.

Attention is now directed to preparation 2109 in Table III. This pupal abdomen received implants of adult corpora allata plus diapausing pupal prothoracic glands. Precisely the same result was observed as after the injection of ecdysone: the pupal abdomen molted and transformed into a second pupal abdomen. In this case it seems necessary to conclude that the corpora allata activated the diapausing prothoracic glands—that, in this sense, a hormone from the corpora allata had substituted for the brain hormone. However, there is no indication in Table III that this corpus allatum hormone can substitute for ecdysone itself.

6. *Tests of adult corpora allata in previously chilled pupae*

The results considered to this point lead to the prediction that adult corpora allata should be uniformly active when tested in previously chilled pupae just prior to the initiation of adult development.

During the past ten years this prediction has been confirmed on a large scale. The experimental series includes ninety-eight preparations in which corpora allata of male and female moths of *Cecropia*, *Polyphemus*, and *Cynthia* were tested in chilled pupae of each of the same three species. All except eight animals gave rise to adults retaining pupal characters. In the eight negative tests the implanted glands had been derived from elderly adults just prior to death.

There was a rough correlation between the number of implanted glands and the degree to which pupal characters were preserved—a finding which will be considered in further detail in the following paper. Moreover, as was true in the earlier experiments on brainless pupae, the effects of the implanted corpora allata were amplified when the host pupae were placed at 15 or 20° C., rather than at 25° C., immediately after the implantation.

The retention of pupal characters was extreme in many of the test animals. As shown in Figure 2, the pupa transformed into a second pupa which showed only traces of adult characteristics. In several experiments performed on *Polyphemus* and *Cecropia*, the secondary pupa molted into a tertiary form. In this case, the pupal characteristics were less prominent after the second molt than after the first.

None of these animals was viable for any prolonged period after transforming into mixed forms. Although the old pupal cuticle became thin and crisp and the ecdysial lines were eroded to the surface, spontaneous escape from the old pupal cuticle occurred only in individuals showing minimal retention of pupal characteristics. All other animals remained enveloped in the old pupal cuticle until they died or were sacrificed.

In many of the individuals the molting process proceeded to a normal terminal phase accompanied by a complete breakdown of the old endocuticle and a partial or complete resorption of the molting fluid. Yet, for some unexplained reason, the insect failed to undertake the vigorous muscular efforts that accompany a normal ecdysis. It did not "try to molt" even though it possessed the nervous and

muscular equipment to do so. The use of forceps was therefore necessary to peel off the old pupal exuviae.

In many individuals it was difficult or impossible to withdraw the lining of the old tracheal tubes through the spiracular openings. Indeed, in the case of *Cecropia*, the larger branches of this old system became stiff and melanized and therefore incapable of being shed. The net effect is that the juvenile hormone is a lethal agent for all these Saturniid pupae.

7. *Inactivity of killed corpora allata*

The high activity recorded for implanted adult corpora allata suggested the possibility that substantial amounts of hormone might be stored within the glands themselves. This prospect was tested in five experiments. In one experiment eight adult *Cecropia corpora allata* were frozen and thawed twice at -40° C. and then implanted into a previously chilled pupa. Normal development ensued.

In four other experiments adult corpora allata, in numbers ranging from 9 to 44, were homogenized in 0.1 ml. of insect Ringer and then introduced into four previously chilled pupae. All four animals developed into normal adult moths. Evidently, little or no hormone is stored in the living gland, for the activity of a single living adult corpus allatum was not duplicated by the implantation of up to forty-four dead glands.

DISCUSSION

1. *Secretion of the juvenile hormone by the adult corpora allata*

The experimental results demonstrate the endocrine activity of the corpora allata of *Cecropia*, *Polyphemus*, and *Cynthia* moths. As is amply evident in Wigglesworth's (1954) recent review, this finding is consistent with the picture presented in all other insects that have been studied in detail including several families of Lepidoptera. In the *Cecropia* silkworm the corpora allata, when removed and tested, are found to be more active in the adult moth than at any other stage in the life history (Williams, unpublished data). Moreover, there is general agreement that at least one of the secretory products of the adult corpora allata is the same juvenile hormone which is secreted weeks or months earlier by the corpora allata of the immature insect. This conclusion was first proposed by Pflugfelder (1938a, 1938b) and Pfeiffer (1945), and will be further documented in the subsequent papers in this series.

2. *The role of the juvenile hormone in adult moths*

We have been unable to detect any function for the corpora allata in the pupal or adult stages of these Lepidoptera. Thus, as we have seen, the corpora allata can be removed from pupae of either sex without disturbing the development of normal, viable, sexually mature moths. These findings are the same as those reported for *Bombyx mori* by Bounhiol (1938) and Fukuda (1944). The present study enlarges the negative evidence by showing that the absence of corpora allata fails to interfere with the maturation of functional gametes and the production of normal offspring.

The situation in the Lepidoptera therefore departs from that described for most other orders of insects where the corpora allata are necessary for the deposition of yolk in the adult female and for the secretory activity of the accessory glands in the adult male (for summary, see Wigglesworth, 1954, pages 77-80). In the Lepidoptera which have been studied, all these functions can go forward in the absence of corpora allata. For the sexual maturation of both males and females all that is required is the presence of prothoracic gland hormone (ecdysone). The brain hormone is also unnecessary for the sexual maturation of these silkworms. Pupae from which the brain, corpora cardiaca, and corpora allata have been removed develop into sexually mature moths after the injection of crystalline ecdysone (Williams, 1954).

Adult Lepidoptera therefore present the paradoxical picture of the presence of highly active corpora allata for which there appears to be no apparent function. However, it is worth recalling that corpora allata have been tested only in species of adult Lepidoptera which are short-lived and unable to feed. In adults of the giant silkworms, as in the commercial silkworm, functional mouth-parts are absent. Consequently, the duration of the adult stage is greatly curtailed: ripe eggs must be ready for oviposition at the time of adult emergence. In short, the absence of mouth-parts has enforced on these short-lived moths a precocious maturation of the gonads during the course of pupal-adult development. Indeed, months before the development of the adult moth, the proteins which later appear in the yolk of the eggs are already present in high concentrations in the blood of the diapausing pupa (Telfer, 1954).

It is among the feeding, long-lived species of adult Lepidoptera that one would anticipate a gonadotropic function for the corpora allata akin to that seen in most other orders of insects. This inference is in accord with the histological studies of Kaiser (1949) on long-lived butterflies of the genus *Vanessa*. Presumably, in the Ephemeroptera and other non-feeding adults one should find the same picture as presented by the Saturniidae.

The absence of functional adult mouth-parts is clearly a secondary affair in the evolution of the Lepidoptera. Indeed, the very same moths contain digestive tracts of normal organization, but of no apparent function. Evidently, the presence of active corpora allata is a memento of a more primitive endocrinological situation.

3. *Biological role of the juvenile hormone*

The juvenile hormone plays no role in the transformation of a pupa into an adult moth. All that is required is that the juvenile hormone be absent throughout the early phases of this transformation (Williams, 1952b). This conclusion is in line with the finding that the corpora allata are inactive throughout the entire pupal stage and during the first two-thirds of adult development (Williams, unpublished data).

A pupa can be supplied with juvenile hormone by the implantation of living, active corpora allata obtained from larvae or adults. However, as demonstrated in the experiments on isolated pupal abdomens (Table III), the juvenile hormone has no effects in the absence of the prothoracic gland hormone, ecdysone. Only when the abdomen is provided with this hormone can one detect any action of

the implanted corpora allata. The outcome is that the pupal abdomen terminates diapause, molts, and transforms into a second pupal abdomen (Fig. 5).

Substantially the same result is seen in experiments performed on brainless diapausing pupae. Here again the implantation of adult corpora allata is inconsequential unless ecdysone is supplied by injection or by the secretory activity of the animal's own prothoracic glands. The juvenile hormone then opposes the transformation of the pupa into an adult moth. The result (Figs. 3 and 4) is a creature showing to varying degrees a retention of pupal characters of the type previously described by Piepho (1952) and Williams (1952b). When the titer of juvenile hormone is high, then one may witness the formation of a *bona fide* second pupal instar—a phenomenon hitherto unknown in any insect (Fig. 2). But, even in the presence of the highest concentrations of juvenile hormone, we have never observed in this material the reappearance of larval characters such as described in *Rhodnius* (Wigglesworth, 1954, 1957, 1958).

4. *Mimicking of brain hormone*

In a certain proportion of brainless diapausing pupae the implantation of active corpora allata causes the termination of diapause and the initiation of adult development. This result is not seen in isolated pupal abdomens or other preparations lacking prothoracic glands. But, as noted in Table III, the developmental reaction becomes possible if an isolated abdomen receives active corpora allata plus inactive prothoracic glands, or active corpora allata plus an injection of ecdysone (Fig. 5). Moreover, in numerous experiments to be described on a later occasion, the development of brainless diapausing pupae has been provoked by the injection of crude or purified extracts of juvenile hormone. Evidently, under certain undefined conditions, a hormonal secretion of the corpora allata can activate the prothoracic glands and, in this sense, mimic the function of the brain hormone. Whether this hormone is the juvenile hormone or some further secretory product of the corpora allata is impossible to state at the present time. A decision on this point will become possible only when the juvenile hormone is isolated and tested in pure form.

The finding that the corpora allata can turn on the prothoracic glands has an obvious bearing on the endocrine control of larval molting. If the corpora allata can activate the pupal prothoracic glands, there is no reason to suppose that they cannot do so in the immature larva.

We begin to see a multiplicity of agencies which can promote the secretion of ecdysone by the prothoracic glands. The brain can turn on the prothoracic glands. Ecdysone can turn on the prothoracic glands (Williams, 1952a, 1954). And, evidently, under certain undefined conditions, so also can the corpora allata. Nature has apparently found it prudent to surround the prothoracic glands by a net-work of controls. The present study suggests that the corpora allata are a part of that net-work.

SUMMARY

1. Juvenile hormone is secreted in high concentration by the corpora allata of the adult *Cecropia* moth.

2. Notwithstanding this fact, the juvenile hormone has no apparent function in the adult moth. Extirpation of the corpora allata in the pupal stage fails to interfere with the production of normal moths whose gametes give rise to normal offspring.

3. The corpora allata are inactive during the entire pupal stage as well as during the first two-thirds of adult development. If active corpora allata are implanted into a pupa just prior to the initiation of adult development, the juvenile hormone acts to oppose the differentiation of the adult moth. Development gives rise to an insect showing a mixture of pupal and adult characters. In the presence of high concentrations of juvenile hormone the pupa molts and transforms into a second pupa showing only traces of adult characters.

4. The biological action of juvenile hormone is seen only in the presence of active prothoracic glands or their secretory product, ecdysone. Isolated pupal abdomens fail to respond to juvenile hormone unless ecdysone is simultaneously present. When both hormones are present, the pupal abdomen terminates diapause, molts, and transforms into a second pupal abdomen.

5. Evidence is presented that the corpora allata secrete a factor which can mimic the brain hormone and activate the prothoracic glands. This finding is considered in relation to the endocrine control of larval molting.

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